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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Vascularized composite allografts (VCA) are transplants containing multiple tissue types (including bone, muscle, skin, nerves and blood vessels), which offer patients restoration of function and form following severe, disabling and disfiguring injury or tissue loss, in circumstances where the results of conventional reconstructive surgery remain unsatisfactory.

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INTRODUCTION

Vascularized composite allografts (VCA) are transplants containing multiple tissue types (including bone, muscle, skin, nerves and blood vessels), which offer patients restoration of function and form following severe, disabling and disfiguring injury or tissue loss, in circumstances where the results of conventional reconstructive surgery remain unsatisfactory. The high incidence of episodes of skin-targeted acute rejection, and the morbidity associated with current immunosuppression regimens, necessary throughout the life of the recipient to prevent rejection, remain significant areas in which improvement would enhance quality of life, improve the risk-benefit ratio of VCA and ultimately expand availability of these procedures to severely injured service men and women, and civilian victims of disabling and disfiguring trauma or disease (Leonard et al, 2013). The objective of the VCA laboratory at the MGH is to develop a clinically-applicable strategy for the induction of immune tolerance of VCAs. The aim of the work supported by this award is to introduce and optimize a protocol for VCA tolerance based on the principle of delayed induction of mixed chimerism in a non-human primate (NHP) model. This approach, in contrast to protocols which have already reached clinical trials for kidney transplantation, permits induction of tolerance in the context of transplantation from deceased donors – a prerequisite for clinical application in VCA. Successful induction of tolerance for VCAs using this protocol in NHPs can be expected to lead to rapid translation into clinical trials.

KEYWORDS

Vascularized composite allograft, vascularized composite allotransplantation, restorative transplantation, transplant tolerance, mixed chimerism, delayed induction of transplant tolerance, non-human primate model.

OVERALL PROJECT SUMMARY

i. Progress against Current Objectives

*****<u>Previous</u> Statement of Work*****

Year 1: Aim 1: To Optimize the Delayed Tolerance Induction Protocol for Vascularized Composite Allotransplantation in a Non-Human Primate Model.

Task	Subtask	Months	Progress
(1.1) TASK 1. Optimize standard immunosuppression (SIS) protocol for upper extremity transplantation in nonhuman primates. (Months 0-12)	(1.1.1) SUBTASK 1. IACUC and ACURO review and approval. (Month 0-3)	Month 0-3	100%
	(1.1.2) SUBTASK 2. Orthotopic upper extremity transplants on SIS protocol (n=3). (Month 3-6)	Month 3-6	Deferred/Award modification requested (See note)
	(1.1.3) SUBTASK 3. Investigate VCA survival, frequency of complications, withdraw SIS at 6 months, document rejection process clinically and histologically. (Month 3-12)	Month 3-12	Deferred/Award modification requested (See note)
	(1.1.4) SUBTASK 4. Summarize optimal immunosuppressive requirements for VCA survival in NHPs. Analyse and summarize data on VCA rejection. Year 1 report. (Month 12)	Month 12	Deferred/Award modification requested (See note)

(1.2) TASK 2. Investigate delayed tolerance induction protocol (DTIP) for upper extremity transplantation in nonhuman primates. (Months 6-18)	(1.2.1) SUBTASK 1. Orthotopic upper extremity transplants on 4 months SIS (n=4). (Months 6-9)	Month 6-9	75%
	(1.2.2) SUBTASK 2. Delayed tolerance induction protocol, wean immunosuppression. (Months 10-13)	Month 10-13	75%
	(1.2.3) SUBTASK 3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 10-18)	Month 10-18	75%
	(1.2.4) SUBTASK 4. Summarize preliminary data/progress on DTIP transplants for inclusion in year 1 report(Month 12)	Month 12	75%
(1.3) TASK 3. Produce LFA-3-IgG for use in Aim 2/Aim 3 (Months 0-24)		Month 0-24	50%

Table 1. Progress against objectives (previous SOW)

Notes:

1. Previous Task 1 Progress/Award Modification Request:

Task 1 Subtasks 2-4 was previously deferred and an award modification request submitted to the GOR for elimination to avoid duplication of work in identifying optimal immunosuppressive doses for this protocol. Animals from this deleted group (3 donors, 3 recipients) have been redistributed to the remaining experimental groups to avoid any negative impact on the significance of our results due to the slightly higher than predicted rate of technical complications in the first year.

*****<u>Current</u> Statement of Work*****

Year 1-2: AIM 1. To optimize the delayed tolerance induction protocol for vascularized composite allotransplantation in a non-human primate model.

(1.1) TASK 1. Investigate delayed tolerance induction protocol (DTIP) for upper extremity transplantation in nonhuman primates. (Months 0-18)	(1.1.1) SUBTASK 1. IACUC and ACURO review and approval. (Month 0-4)	Month 0-4	100%
	(1.1.2) SUBTASK 2. Order and take delivery of first cohort of non-human primates. (Month 5-6)	Month 5-6	100%
	(1.1.3) SUBTASK 3. Orthotopic upper extremity transplants on 4 months SIS (n=4). (Months 6-9)	Month 6-9	100%
	(1.1.4) SUBTASK 4. Delayed tolerance induction protocol, wean immunosuppression. (Months 10-13)	Month 10-13	100%

	(1.1.5) SUBTASK 5. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 10-18)	Month 10-18	100%
	(1.1.6) SUBTASK 6. Summarize preliminary data/progress on DTIP transplants for inclusion in year 1 report (Month 12)	Month 12	100%
(1.2) TASK 2. Produce LFA-3-IgG for use in Aim 2/Aim 3 (Months 0-24)		Month 0-24	50%

Year 2-3: AIM 3. To investigate the effect of combined T memory cell inhibition and T regulatory cell up regulation on the delayed induction of VCA tolerance

(2.1) TASK 1. Investigate effect of Tmem inhibition on delayed induction of VCA tolerance (Months 12-24)	(2.1.1) SUBTASK 1. Orthotopic upper extremity transplants on 4 months SIS (n=4) (Months 12-15)	Month 12-15	Award modification requested (See note)
	(2.1.2) SUBTASK 2. Delayed tolerance induction protocol + CTLA4-Ig/LFA-3-IgG. (Months 16-19)	Month 16-19	Award modification requested (See note)
	(2.1.3) SUBTASK 3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 16-24)	Month 16-24	Award modification requested (See note)
	(2.1.4) SUBTASK 4. Summarize and report data on effect of Tmem inhibition on delayed induction of VCA tolerance for year 2 report (Month 24)	Month 24	Award modification requested (See note)
(2.2) TASK 2. Investigate effect of Treg up-regulation on delayed induction of VCA tolerance (Months 16-24)	(2.2.1) SUBTASK 1. Orthotopic upper extremity transplants on 4 months SIS (n=4) (Months 17-20)	Month17-20	Award modification requested (See note)
	(2.2.2) SUBTASK 2. Delayed tolerance induction protocol + a-IL-6R (Months 20-24)	Month 20-24	Award modification requested (See note)
	(2.2.3) SUBTASK 3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 17-24)	Month 17-24	Award modification requested (See note)
	(2.2.4) SUBTASK 4. Summarize and report data on effect of Treg upregulation on delayed induction of VCA tolerance for year 2 report (Month 24)	Month 24	Award modification requested (See note)

(3.1) TASK 1. Investigate effect of combined Tmem inhibition and Treg up regulation on delayed induction of VCA tolerance (Months 22-36)	(3.1.1) SUBTASK 1. Orthotopic upper extremity transplants on 4 months SIS (n=4) (months 22-25)	Month 22-25	Award modification requested (See note)
	(3.1.2) SUBTASK 2. Heterotopic partial face transplants on 4 months SIS (n=4) (Months 25-28)	Month 25-28	75%
	(3.1.3) SUBTASK 3. DTIP with combined Tmem inhibition/Treg upregulation. (Months 26-32)	Month 26-32	Award modification requested (See note)
	(3.1.4) SUBTASK 4. Investigate durability of chimerism, VCA survival, frequency of complications (eg GvHD) and in vitro immune status (Months 26-36)	Month 26-36	Award modification requested (See note)
	(3.1.5) SUBTASK 5. Summarize and report data on effect of combined Tmem inhibition/Treg upregulation on delayed induction of VCA tolerance for year 3 report (month 36)	Month 36	Award modification requested (See note)
	(3.1.6) SUBTASK 6. Complete data analysis, prepare final (year 3) report, prepare manuscripts for submission	Month 36	Award modification requested (See note)

Table 2. Progress against objectives (current SOW)

Notes:

1. Award Modification Request:
Following the submission of our award modification request in February 2014 and approval in September 2014, 2 more upper extremity VCAs were performed but both were lost due to technical failures on POD 0 (M1214) and POD 22 (M4113). In view of this higher than expected technical complication rate in this model, it was decided to progress directly to the heterotopic partial face transplant model in Aim 3 Task 1 Subtask 2. We have had a much better technical success rate thus far (4/5, 80%) and have made important new findings necessitating a further modification request as detailed in subsequent sections of this report and in Appendices 1 and 2.

ii. Results

Investigation of delayed tolerance induction protocol for upper extremity transplantation in NHPs

M4113

Transplantation proceeded without complication and the modified transplant technique based on our experience from Year 1 of this award and our clinical experience (Eberlin et al, 2014), led us to place the arterial anastomosis more proximally in the arm to achieve greater vessel caliber. This appeared to have been successful as the hand reperfused quickly following completion of the microsurgical anastomosis. However, the transplanted upper extremity VCA gradually started to demarcate and necessitated sacrifice and removal from the study at POD 22. We believe that this was a technical failure due to venous congestion which reflects both a threshold venous drainage through anastomosis with the cephalic vein, and the dependent position in which the NHPs tend to hold their arms.

M1214

The animal was the recipient of a hand transplant but was sacrificed intra-operatively. The transplanted hand was assessed prior to completion of the procedure – there was no bleeding observed on finger prick test of the VCA, muscle bellies appeared dusky and poorly perfused; the anastomoses were patent, but inflow was sluggish. Despite primary revision of the arterial anastomoses, there was still no improvement observed. Further extensive attempts to optimize perfusion (temperature, increased blood pressure, analgesia, vasopressors etc) were all to no avail and the decision was taken that recovery of the animal would be futile in the absence of clear signs of perfusion. The NHP was thus euthanized on table with IV 100mg/kg sodium pentobarbital and removed from the study.

By this point in the workflow for this award, we had encountered 4 out of 5 technical failures with this model and hence, the decision was taken to proceed with the use of the face transplant model from Aim 3 Task 1 Subtask 2 instead.

Investigation of delayed tolerance induction protocol for partial face transplantation in NHPs

Using our previously established screening and selection algorithm for donor and recipient NHP pairs, 5 face transplants (Barth et al, 2009) have been performed thus far during the period covered by the current annual report for this award.

M6014

Technically successful surgery and the animal survived for 107 days before euthanasia due to the weight loss criteria while on the 4 month delay period as per the original SOW. Despite multiple, various attempts including titration of immunosuppression dosages (MMF, tacrolimus) and various adjunctive medications including the use of mirtazapine after consultation with the veterinarian, there was no improvement in the NHP's diet in the hopes of promoting weight gain. At necropsy, the transplanted face VCA had a well-vascularized bone marrow component that could be clearly seen; the NHP Pathology report showed that the animal had developed post-transplant lymphoproliferative disorder (PTLD). *In vitro* investigations revealed that the animal's T cells in the VCA were ~85% recipient on POD 28 biopsy and ~100% at all protocol biopsy timepoints thereafter. The animal also experienced 3 acute rejection episodes on POD 28, 48 and 60 (Figure 1A, B, C) that were all successfully treated and resolved with steroid pulses and at the time of euthanasia on POD 107, showed neither clinical nor histological signs of rejection in the VCA (Figure 1D).

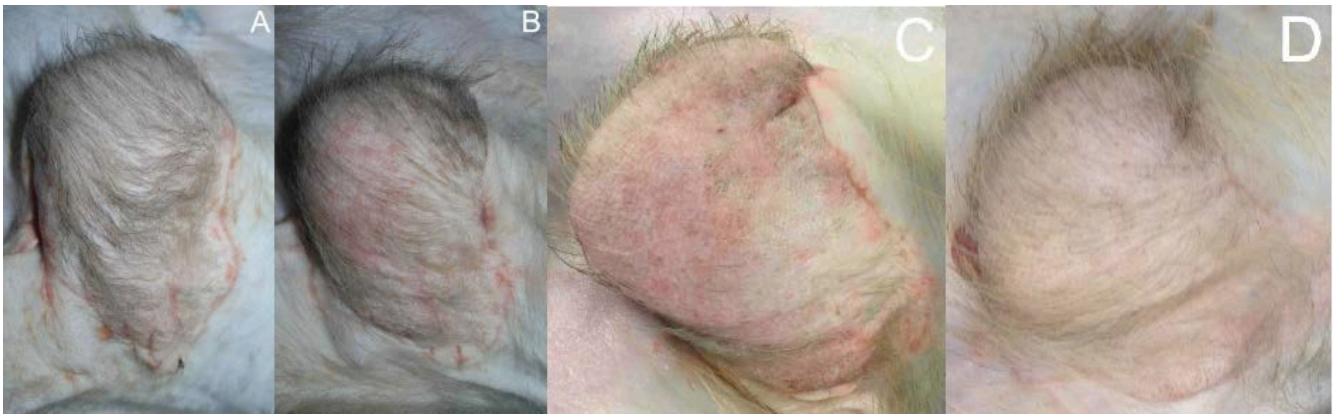

Figure 1. Clinical appearance of heterotopic partial face transplant in M6014. Acute rejection episodes on (A) POD 28, (B) POD 48, (C) POD 60; (D) clinically viable VCA on POD 107 before euthanasia.

M7114

During closure on the day of surgery, the VCA showed a slight ooze and the decision was made to place a penrose drain (Figure 2A) to promote drainage and prevent a hematoma from building up. The next day the VCA had stopped oozing but was cold to touch. Over the ensuing days, the flap demarcated and could not be salvaged due to the onset of necrotic, full-thickness loss (Figure 2B, 2C). The animal was therefore euthanized on POD 9 and venous congestion due to a technical failure appeared to be the primary cause of the flap loss and removal of the animal from the study.

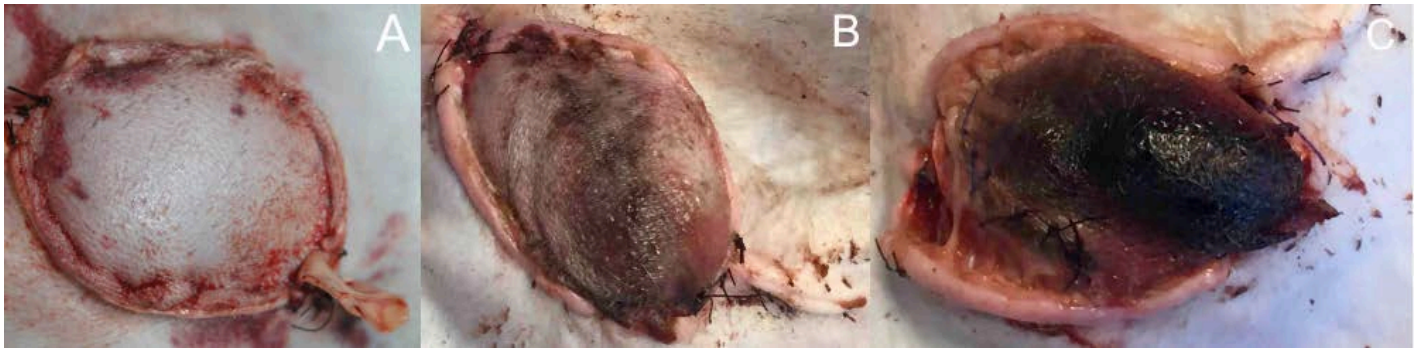

Figure 2. Clinical appearance of heterotopic partial face transplant in M7114. (A) POD 1, after placement of penrose drain to promote drainage, (B) demarcation of VCA consistent with venous congestion on POD 6, (C) full-thickness loss and necrosis of VCA on POD 9 requiring termination of experiment.

M6714

Technically straightforward procedure and is currently >60 days post-transplant with neither clinical nor histological evidence of rejection (Figure 3). The animal developed a suspicious looking lesion around POD 21 on the VCA itself (nearer to the inferior aspect) which was biopsied and treated presumptively as an episode of acute rejection with steroid boluses. Histopathology was reported as minimal inflammation with mild necrosis and without evidence of acute rejection on the Banff scale; serum allo-antibody was negative. Overall, given the clinical picture, it was most likely to be in keeping with mild trauma from scratching. The animal is otherwise clinically well, maintaining its weight, and is currently on triple immunosuppression while awaiting ACURO approval to initiate conditioning in preparation for bone marrow transplantation (BMT).

In vitro investigations showed a slight but insignificant anti-donor response by MLR assay, which is not unexpected as the animal is currently on immunosuppression and mixed chimerism had not been induced. Protocol VCA biopsies at POD 30 and 60 revealed that T cells in the VCA were ~100% recipient-origin by POD 30 and this level remained consistent thereafter, which supports our amendment request to initiate conditioning for BMT as soon as possible i.e. once it is clinically stable and has had sufficient time to recover from surgery, and there are no *in vitro/in vivo* evidence of rejection, infection, systemic compromise.

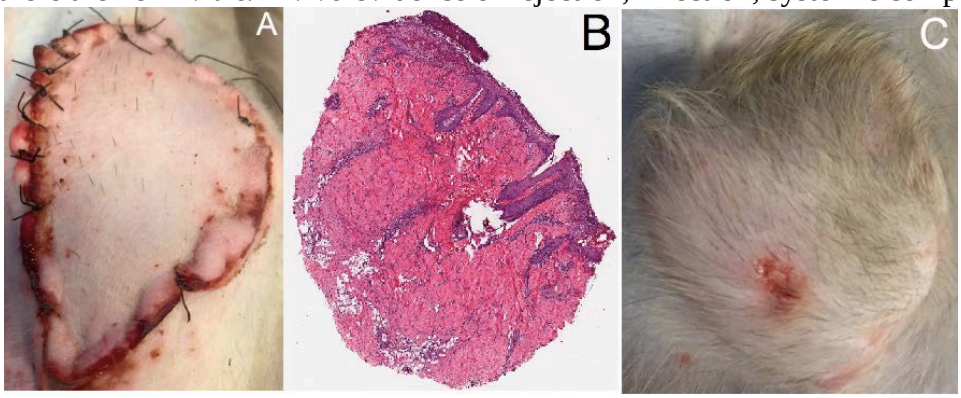

Figure 3. Clinical appearance of heterotopic partial face transplant in M6714. (A) POD 2, where VCA is warm, viable and recovering well from surgery, (B) H&E biopsy at POD 21 was negative for acute rejection, (C) POD 59, showing site of previous biopsy on the VCA which is almost healed by secondary intention; note also the growth in hair of the VCA.

M6914

Technically complicated procedure. Intra-operative blood loss of the donor was greater than usual and for the first time ever, we could not get sufficient donor blood ready for intra-operative transfusion of the recipient. It was also noted that the recipient animal had a prolonged run of low systolic blood pressure in the 50s that did not respond to intravenous fluid or vasopressor (dopamine) administration following increased sedation with isoflurane and ketamine that was necessitated intra-operatively because the animal was waking up mid-way through the microsurgical anastomosis. Upon completion of the transplant, extubation and recovery from anesthesia, the animal went into asystolic collapse and could not be revived despite 5 minutes of CPR.

Upon internal review, the cause of loss was attributed to a combination of anesthetic complication and underestimation of the intra-operative blood loss. We have since mandated, internally, insertion of an intra-arterial femoral line for closer monitoring of the donor's intra-operative blood pressure, and to have a vascular access port inserted into the recipient prior to commencement of the recipient's part of the surgery so as to enable blood gas and CBC monitoring as required.

M6514

Uncomplicated procedure. This animal is the recipient of a partial heterotopic face transplant which is now 14 days post transplant. Animal is being maintained on triple immunosuppression and is awaiting conditioning for BMT on POD 60 (as per our amendment request). No episodes of acute rejection have been encountered thus far and the NHP is otherwise clinically well and with a viable VCA (Figure 4). This is our fifth face transplant and demonstrates that we are now able to reliably and consistently perform this procedure without major complications.

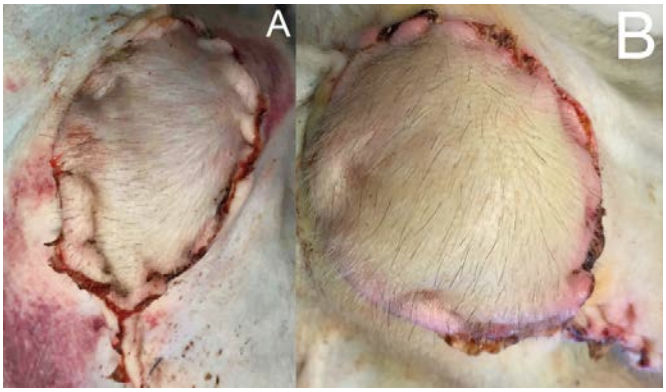

Figure 4. Clinical appearance of heterotopic partial face transplant in M6514. (A) POD 2, with some post-operative bruising but the VCA was otherwise not compromised, (B) POD 6, swelling and bruising have markedly resolved, confirming the technical success of the procedure.

Flow cytometric analysis of cutaneous leukocytes during delay period

As previously described, our laboratory is now equipped with the ability to investigate the infiltrating leukocyte populations in NHP models of VCA. Remarkably, in both face transplant NHPs that have survived beyond POD 30, results of VCA and host skin biopsies are consistent with the findings in our work in upper extremity NHP models in our first year of work i.e. an almost complete turnover of cutaneous leukocytes to that of recipient origin by POD 30 (Figure 3). This result mirrors that of our clinical human hand transplant patient who is currently on triple immunosuppression and has demonstrated a near complete turnover of skin resident leukocytes to recipient origin by 18 months post-operative.

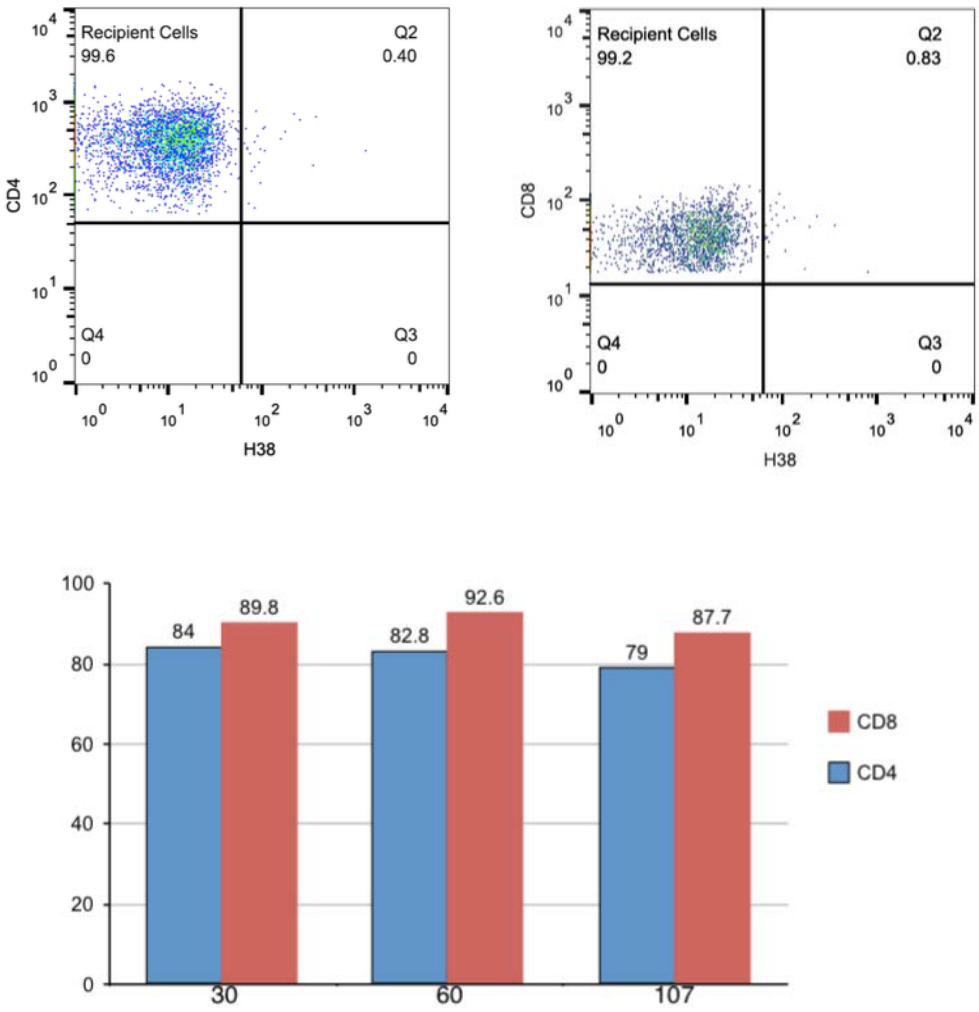

Figure 3. Representative data from M6014. (*Top*) Near complete turnover of resident T cells in VCA dermis to that of recipient-origin on flow cytometric analysis, (*Bottom*) percentage of CD4 and CD8 T cells in VCA dermis at POD 30, 60 and 107 (experimental end-point) demonstrating consistent numbers throughout.

iii. Changes/Problems

Award Modification Request

We have submitted an award modification request on October 2, 2015 seeking approval to modify the delay period in all remaining experimental groups to 2 months from the original 4 months. This request originated following the clinical course of M4213 (upper extremity VCA in the first year) and M6014, when it became clear that acute rejection episodes tended to occur between 1 and 3 months post-VCA and due to a combination of high-dose immunosuppression required in this species and the immunogenicity of the skin component of VCA, it would be prudent to initiate recipient conditioning for bone marrow transplantation sooner to induce mixed chimerism for the withdrawal of immunosuppression.

Technical Failures

We attribute the technical failure of M7114 to the initial learning curve, and have learnt invaluable lessons from the unfortunate loss of M6914. We have shown that the changes that we have implemented – (i) invasive monitoring of blood pressure in the donor NHP so as to prevent on-table exsanguination from VCA harvest, will provide for an immediate source of whole blood that can be readily used for transfusion, if necessary, for

the recipient, and (ii) immediate placement of a vascular access port for intra-operative monitoring of blood gas and CBC, which will provide the anesthetic and surgical teams with sufficient information of the recipient's clinical status to minimize the risk of surgery-related complications by enabling early transfusion and closer titration of intravenous fluids and/or vasopressors as necessary to ensure a smooth operative course and recovery. This is evident with a much less stormy procedure for M6514.

iv. Discussion

Translation of novel protocols for the immunologic management of patients undergoing restorative transplantation procedures requires the highest level of preclinical scrutiny, and demonstration of the safety and efficacy of proposed protocols in stringent model systems. NHP models are recognized to play a key role in this translational pathway. We have developed a NHP upper extremity transplantation model, and during this reporting period have commenced work utilizing this model to the investigation of delayed induction of transplant tolerance; a paradigm with potential application not only to reconstructive transplantation, but to transplantation of solid organs from deceased donors, which has already proven successful in similar NHP studies of kidney transplantation (Yamada et al, 2012).

The successful execution of experiments of this type is critically dependent on the availability of suitable, high quality experimental animals from reliable vendors. We have established a reliable supply chain with Charles River Laboratories, and the development of a screening and selection algorithm to facilitate selection, shipping and quarantine of suitable animals in a timely manner in preparation as we progress through the experiments outlined in the statement of work.

While our award modification request is pending we have proceeded with further NHP face transplants to achieve the minimum numbers required for statistical significance of the experimental arm. Overall, we do not envisage any problems with completing the described work within the projected time period of this award.

With regard to experimental outcomes during this reporting period, further technical losses of hands transplanted to M4113 and M1214 was highly disappointing but we are encouraged with our technical success with the face transplant model thus far. While we work towards completion of the SOW for face transplants, we will take into consideration the intra-operative modifications that we have developed for application to further hand transplants in view of the potential differences in immunological outcomes between hand and face transplants due to innate differences between the skin of the facial and upper extremity regions. Certainly, if the face transplant model proves more reliable and consistent, we will not hesitate to request for approval for all remaining experiments to be performed using it. If so, we will also consider switching the hand transplant model from NHP (cynomolgus macaques) to baboons should the upper extremity VCA model remain important to our work because of the larger physical size of baboons which would translate to larger caliber vessels for a greater likelihood of technical success after VCA procedures.

As described in our previous annual report, incidences of acute rejection prior to conditioning and attempted tolerance induction is to be expected in this model. However, in view of the severity of some of these rejection episodes leading to irreversible VCA loss on POD 51, plus the systemic complications (e.g. PTLD) encountered during the delay period of 4 months, we believe that shortening to 2 months will allow sufficient time for adequate resolution of the peri-transplant inflammatory milieu whilst not subjecting the animal to the systemic sequelae of a prolonged period of high dose immunosuppression that may now, in retrospect, not be entirely necessary. This will hopefully enable BMT to be carried out sooner with engraftment and thus, stable mixed chimerism to allow complete withdrawal of immunosuppression.

In terms of *in vitro* studies, the finding that skin resident leukocytes undergo an almost complete turnover by POD 30 in NHPs reflects the importance of initiating conditioning for BMT at an earlier time point rather than at 4 months as described in the original SOW. In addition, this finding mirrors that of our clinical hand transplant patient and adds further credence to the utility of our NHP model in our VCA studies. Overall, these findings are consistent with our previous studies in a porcine model (Leonard et al, 2014) and it is hoped that important mechanistic insights can be derived as this project progresses and perhaps, the eventual development of novel topical therapeutic targets at the skin immune system.

KEY RESEARCH ACCOMPLISHMENTS

The following represent key accomplishments of this research during this reporting period:

- *Optimization of the delayed tolerance induction protocol in non-human primates.*

    - By reducing the delay period from the original 4 months to 2 months, we are confident that the number of acute rejection episodes can be minimized or eliminated completely even. This is particular pertinent in view of our previous experiments where acute rejection led to irreversible VCA loss. In addition, decreasing the delay period will also reduce the overall duration of exposure to high-dose immunosuppression and the attendant risks of related complications such as PTLD and cachexia which can lead to irreversible weight loss and premature termination of the experiment.

- *Optimization of the heterotopic partial face transplant procedure in non-human primates.*

    - Following the unfortunate loss of M6914 after recovering from anesthesia, we have implemented closer, invasive intra-operative monitoring of blood pressure through the placement of an intra-arterial line in the femoral artery. Aggressive peri-operative preparation in the form of irradiated whole blood from the exsanguinated donor and recipient-typed blood from other experimental animals has ensured that blood transfusions are readily available as necessary for intra-operative support.

CONCLUSION

The induction of transplant tolerance for reconstructive transplantation would be of considerable benefit to civilian victims of disabling and disfiguring tissue loss, and of significant importance to military victims of upper extremity and/or craniofacial trauma. Currently, the necessity of life-long immunosuppression and regular medical monitoring would prevent recipients of restorative transplants (such as hand or face transplant) from returning to active duty, but a safe and effective protocol for induction of transplant tolerance holds the potential to fundamentally change this paradigm.

Introduction of novel protocols of this type to clinical practice clearly requires the highest degree of rigor during pre-clinical testing prior to translation to clinical trial. Consistent with this, research in large animal models is challenging, and unsurprisingly we have faced a number of challenges during this reporting period. However, despite this, progress toward our aims has been steady, the challenges met have been carefully analyzed and corrective action plans determined and implemented. Furthermore, improvements in our ability to isolate and analyze immunologically active cells from small volume skin biopsies is an important development which can be expected to facilitate significant insights into the mechanisms operational in VCA acceptance under immunosuppression, rejection and tolerance, as this project continues.

Overall, despite the obvious challenges encountered, we are encouraged by our overall progress during this reporting period, and expect to proceed apace during year three of this award. We expect that our analysis of the technical failures encountered, the immuosuppression-related complications encountered during the original four month delay period, and the lessons learned from this analysis, will facilitate rapid progress in future experiments.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

1. Lay Press:
Nothing to report

2. Peer-Reviewed Scientific Journals:

Work supported by this award, in combination with our preliminary studies presented as preliminary data in the application for this award, has so far resulted in the acceptance of one peer-reviewed manuscript for publication:

Leonard DA, Harrison P, Albritton A, Shanmugarajah K, Mastroianni M, Lofgren S, Winter J, Kurtz JM, Cetrulo CL Jr. Upper Extremity Transplantation in Non-Human Primates: An Orthotopic Model for Translational Research. *Vascularized Composite Allotransplantation* 2015;2(1):17-25.

3. Invited Articles:
Nothing to report.

4. Abstracts:

Leonard DA, Mallard C, Shanmugarajah K, Mastroianni M, Albritton A, Powell H, Kurtz JM, Cetrulo CL Jr. Development of an Orthotopic Upper-Extremity Vascularized Allotransplant Model in Non-Human Primates. Poster Presentation. Harvard Medical School Surgical Research Symposium. Boston, Massachusetts. May 2014.

Leonard DA, Mallard C, Shanmugarajah K, Mastroianni M, Albritton A, Powell H, Kurtz JM, Cetrulo CL Jr. Development of an Orthotopic Upper-Extremity Vascularized Allotransplant Model in Non-Human Primates. *Oral Presentation. New England Society of Plastic & Reconstructive Surgeons*. Sebasco, Maine. June 2014.

Leonard DA, Mallard C, Shanmugarajah K, Mastroianni M, Albritton A, Powell H, Kurtz JM, Cetrulo CL Jr. Development of an Orthotopic Upper-Extremity Vascularized Allotransplant Model in Non-Human Primates. *Poster Presentation. Military Health System Research Symposium*. Fort Lauderdale, Florida. August 2014.

Leonard DA, Mallard C, Shanmugarajah K, Mastroianni M, Albritton A, Powell H, Kurtz JM, Cetrulo CL Jr. Development of an Orthotopic Upper-Extremity Vascularized Allotransplant Model in Non-Human Primates. *Poster Presentation. American Society for Reconstructive Transplantation Biennial Meeting.* Chicago, Illinois. November 2014.

INVENTIONS, PATENTS AND LICENSES
Nothing to report.

REPORTABLE OUTCOMES

1. Development of an orthotopic upper-extremity vascularized allotransplant model in non-human primates

2. Validation of techniques for isolation and flow cytometric analysis of skin-resident leukocyte populations in non-human primate skin

3. Optimization of the delayed tolerance induction protocol in non-human primates by reducing the delay period from four to two months.

OTHER ACHIEVEMENTS

Nothing to report.

REFERENCES

Barth RN, Bluebond-Langner R, Nam A, et al. Facial subunit composite tissue allografts in nonhuman primates: I. Technical and immunosuppressive requirements for prolonged graft survival. Plast Reconstr Surg 2009;123(2):493-501

Eberlin KR, Leonard DA, Austen WG et al, The volar forearm fasciocutaneous extension: a strategy to maximize vascular outflow in post-burn injury hand transplantation. Plast. Reconstr. Surg 2014;134:731

Leonard DA, Kurtz JM, Cetrulo CL Jr. Vascularized composite allotransplantation: towards tolerance and the importance of skin specific immunobiology. Curr Opin Organ Transplant 2013;18:645-51

Leonard DA, Kurtz JM, Mallard C et al. Vascularized composite allograft tolerance across MHC barriers in alarge animal model. Am J Transplant 2014;14:343-55

Yamada Y, Boskovic S, Aoyama A, et al. Overcoming memory T cell responses for induction of delayedtolerance in nonhuman primates. Am J Transplant 2012;12:330-40

APPENDICES

Appendix 1: Award Modification Request (Pending Approval)

Appendix 2: Modified Statement of Work (Pending Approval)

Appendix 3: Year 2 Quad Chart

MASSACHUSETTS GENERAL HOSPITAL
HARVARD MEDICAL SCHOOL

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Attending Plastic and Reconstructive Surgeon
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Senior Investigator/Head, Vascularized Composite Tissue Allotransplantation Laboratory
Transplantation Biology Research Center

October 1, 2015

Gay Hayden
US Army Medical Research Acquisition Activity
820 Chandler Street
Fort Detrick, MD 21702-5014
301-619-9883; gay.c.hayden.civ@mail.mil

Re: W81XWH-13-2-0062, MR120034P5
Tolerance in Nonhuman Primates by Delayed Mixed Chimerism
PI: Curtis L. Cetrulo, Jr., M.D., FACS

Dear Ms. Hayden:

We seek your approval to make a modification regarding our award and corresponding animal protocol (2013N000191: *VCA Tolerance in Non-Human Primates by Delayed Mixed Chimerism (RTR)*). In this amendment (AME6), based on preliminary results of our NHP experiments thusfar, we propose to amend the period of delay in the DTIP from 4 months (originally) to 2 months prior to bone marrow transplantation. This will depend on a combination of factors including clinical stability of the NHP based on overall health status (activity, diet) and viability of the flap. This will reduce the overall duration of immunosuppression to avoid complications such as repeated acute rejection episodes and PTLD and allow us the best chance of getting to recipient conditioning and transplantation of donor bone marrow for generating mixed chimerism for VCA tolerance. Please note this amendment will not require any changes to our budget.

Sincerely,

Curtis L. Cetrulo, Jr., MD, FACS

Theresa Vallese
Senior Grants Administrator
Partners Research Management
tvallese@partners.org

Cc: ACURO (usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil)
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Dr. Michael Romanko (michael.j.romanko.ctr@mail.mil)

Year	AIM	TASK	SUBTASK	MONTH(S)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Year 1	AIM 1. To optimize the delayed tolerance induction protocol for vascularized composite allotransplantation in a non-human primate model.	(1.1) TASK 1. Investigate version 1 delayed tolerance induction protocol (DTIP) for upper extremity transplantation in nonhuman primates. (Months 0-18)	(1.1.1) SUBTASK 1. IACUC and ACURO review and approval. (Month 0-4)	Month 0-4	X	X	X	X																																
			(1.1.2) SUBTASK 2. Order and take delivery of first cohort of non-human primates. (Month 5-6)	Month 5-6					X	X																														
			(1.1.3) SUBTASK 3. Orthotopic upper extremity transplants on ~~4~~ 2 months SIS (n=4). (Months 6-9)	Month 6-9						X	X	X	X	~~X~~	~~X~~	~~X~~																								
			(1.1.4) SUBTASK 4. Delayed tolerance induction protocol, wean immunosuppression. (Months 10-13)	Month 10-13										X	X	X	X																							
			(1.1.5) SUBTASK 5. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 10-18)	Month 10-18										X	X	X	X	X	X	X	X																		
			(1.1.6) SUBTASK 6. Summarize preliminary data/progress on DTIP transplants for inclusion in year 1 report(Month 12)	Month 12												X																								
Year 1 -2		(1.2) TASK 2. Produce LFA-3-IgG for use in Aim 2/Aim 3 (Months 0-24)		Month 0-24	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X											
Year 2	AIM 2. To investigate the effect of T memory cell inhibition and in-vivo T regulatory cell up regulation on the delayed induction of VCA tolerance.	(2.1) TASK 1. Investigate effect of Tmem inhibition on delayed induction of VCA tolerance (Months 12-24)	(2.1.1) SUBTASK 1. Orthotopic upper extremity transplants on ~~4~~ 2 months SIS (n=4) (Months 12-15)	Month 12-15												X	X	X	X																				
			(2.1.2) SUBTASK 2. Delayed tolerance induction protocol + CTLA4-Ig/LFA-3-IgG. (Months ~~16-18~~ 14-17)	Month ~~16-18~~ 14-17														X	X	X	X	~~X~~	~~X~~																
			(2.1.3) SUBTASK 3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months ~~16-24~~ 14-23)	Month ~~16-24~~ 14-23														X	X	X	X	X	X	X	X	X	X	~~X~~											
			(2.1.4) SUBTASK 4. Summarize and report data on effect of Tmem inhibition on delayed induction of VCA tolerance for year 2 report (month 24)	Month 24																								X											
		(2.2) TASK 2. Investigate effect of Treg up-regulation on delayed induction of VCA tolerance (Months 16-24)	(2.2.1) SUBTASK 1. Orthotopic upper extremity transplants on ~~4~~ 2 months SIS (n=4) (Months ~~17-20~~ 15-18)	Month ~~17-20~~ 15-18															X	X	X	X	~~X~~	~~X~~															
			(2.2.2) SUBTASK 2. Delayed tolerance induction protocol + a-IL-6R (Months ~~20-24~~ 17-20)	Month ~~20-24~~ 17-20																	X	X	X	X	~~X~~	~~X~~	~~X~~	~~X~~											
			(2.2.3) SUBTASK 3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression. (Months 17-24)	Month 17-24																	X	X	X	X	X	X	X	X											
			(2.2.4) SUBTASK 4. Summarize and report data on effect of Treg upregulation on delayed induction of VCA tolerance for year 2 report (month 24)	Month 24																								X											
Year 2-3	AIM 3. To investigate the effect of combined T memory cell inhibition and T regulatory cell up regulation on the delayed induction of VCA tolerance	(3.1) TASK 1. Investigate effect of combined Tmem inhibition and Treg up regulation on delayed induction of VCA tolerance (Months 22-36)	(3.1.1) SUBTASK 1. Orthotopic upper extremity transplants on ~~4~~ 2 months SIS (n=4) (months 22-25)	Month 22-25																						X	X	X	X										
			(3.1.2) SUBTASK 2. Heterotopic partial face transplants on ~~4~~ 2 months SIS (n=4) (Months 25-28)	Month 25-28																									X	X	X	X							
Year 3			(3.1.3) SUBTASK 3. DTIP with combined Tmem inhibition/Treg upregulation. (Months ~~26-32~~ 24-30)	Month ~~26-32~~ 24-30																									X	X	X	X	X	X	X	~~X~~	~~X~~			
			(3.1.4) SUBTASK 4. Investigate durability of chimerism, VCA survival, frequency of complications (eg GvHD) and in vitro immune status (Months ~~26-36~~ 24-36)	Month ~~26-36~~ 24-36																									X	X	X	X	X	X	X	X	X	X	X	X
			(3.1.5) SUBTASK 5. Summarize and report data on effect of combined Tmem inhibition/Treg upregulation on delayed induction of VCA tolerance for year 3 report (month 36)	Month 36																																			X
			(3.1.6) SUBTASK 6. Complete data analysis, prepare final (year 3) report, prepare manuscripts for submission	Month 36																																			X

Tolerance in Nonhuman Primates by Delayed Mixed Chimerism

Log #: 120034P5

Award #: W81XWH-13-2-0062

PI: Curtis L. Cetrulo, Jr., M.D., FACS **Org:** Massachusetts General Hospital **Award Amount: $1,299,976**

Study/Product Aim(s)

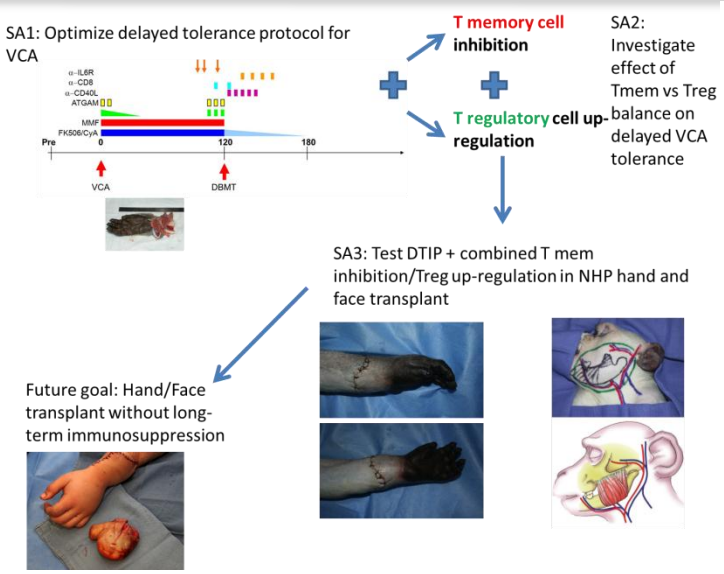
SA1: To optimize the delayed tolerance induction protocol for vascularized composite allotransplantation in a non-human primate model

SA2: To investigate the effect of T memory cell inhibition and in-vivo T regulatory cell up regulation on the delayed induction of VCA tolerance

SA3: To investigate the effect of combined T memory cell inhibition and T regulatory cell up-regulation on the delayed induction of VCA tolerance

Approach

Optimization of delayed tolerance induction protocol in preclinical NHP model of hand transplantation. Delayed tolerance has been successfully induced for organ transplantation in our center; in Aim 1 we will optimize this protocol for VCA. In aim 2 we will investigate tipping balance of negative memory cell and protective, regulatory cell function in favor of tolerance. In aim 3 we will combine these approaches and test in both hand and face transplant models.



Accomplishment: Development of optimal delayed tolerance induction protocol (DTIP) for hand and face transplantation. Successful implementation of this protocol might permit hand/face transplant without lifelong medication.

Activities CY	13	14	15	16
1.1. Investigate version 1 DTIP for hand transplantation in NHPs				
1.2. Production of LFA-3-IgG for aim 2/3 use				
2.1. Investigate effect of Tmem inhibition on hand transplants in NHPs on DTIP				
2.2 Investigate effect of Treg up-regulation on hand transplants in NHPs on DTIP				
3.1. Investigated combined Tmem inhibition/Treg up-regulation on hand and face transplant in NHPs				
Estimated Budget ($)	27,630	388,837	647,063	236,446

Updated: October 14th 2015

Goals/Milestones

CY14 Goals

- ☑Investigate delayed tolerance induction protocol for upper extremity transplantation in NHPs
- ☑Commence production of LFA-3-IgG
- ☑Determine efficacy of basic DTIP for VCA in NHPs

CY15 Goals

- Investigate effect of Tmem inhibition on DTIP for NHP VCAs
- Investigate effect of Treg up-regulation on DTIP for NHP VCAs

CY16 Goals

- Test combined Tmem inhibition/Treg up-regulation on DTIP for VCAs
- Conclude data analysis, prepare reports and manuscripts

Comments/Challenges/Issues/Concerns

- Face transplant model very successful and reproducible
- Technical improvements with upper extremity transplant model under investigation

Budget Expenditure to Date

Projected Expenditure: $885,590

Actual Expenditure: $885,590